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Steroidal Sapogenins XXXIX.

Occurrence and Isolation of Gentrogenin and Correllogenin from *Dioscorea spiculiflora*

After Kendall and Hench first announced the dramatic antiarthritic effects of cortisone in 1948, teams of plant explorers and chemists from various laboratories made great efforts to find suitable plant steroid precursors for this valuable drug. In 1947, Marker and Lopez had reported the isolation of botogenin from *Dioscorea mexicana* tubers (1). Since botogenin was reported to have both the 12 ketone and the $\Delta^{5(6)}$ unsaturated bond, it would have been a very desirable precursor. In subsequent years it has been the object of assiduous searches both by our group and teams from other laboratories, with uniformly negative results. Recently we announced the discovery of two new sapogenins, gentrogenin and correllogenin (2, 3). Gentrogenin and correllogenin have the same structure as those assigned to botogenin and neobotogenin,

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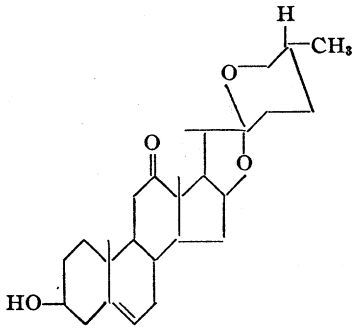
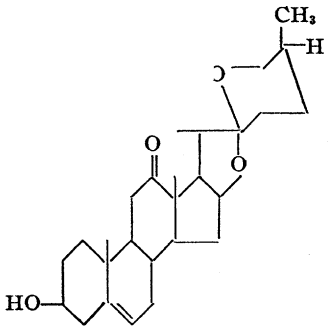


Gentrogenin



Correllogenin

(A) Ground *Dioscorea spiculiflora* tubers

(B) Isopropyl alcohol extraction

Extract | Marc (discard)

(C) Concentrate until aqueous

Extract with *n*-butanol

Butanol layer | Aqueous layer (discard)

(D) Concentrate

(E) Hydrolyze with 2 *N* HCl

(F) Filter

Precipitate | Filtrate (discard)

(G) Reflux in mixture of methanol-potassium hydroxide-benzene

(H) Filter

Precipitate

(K) Extract with benzene

Filter

Precipitate (discard) | Benzene filtrate

Filtrate

Add water (I)

Aqueous layer (J) | Benzene layer (J)

Extract with benzene filtrate (L)

combine

Aqueous layer (discard) | Benzene layer

Concentrate

(M) Crude sapogenin mixture

(N) Dissolve in chloroform

(O) Chromatograph on Florisil

Chloroform eluate

(P) Concentrate

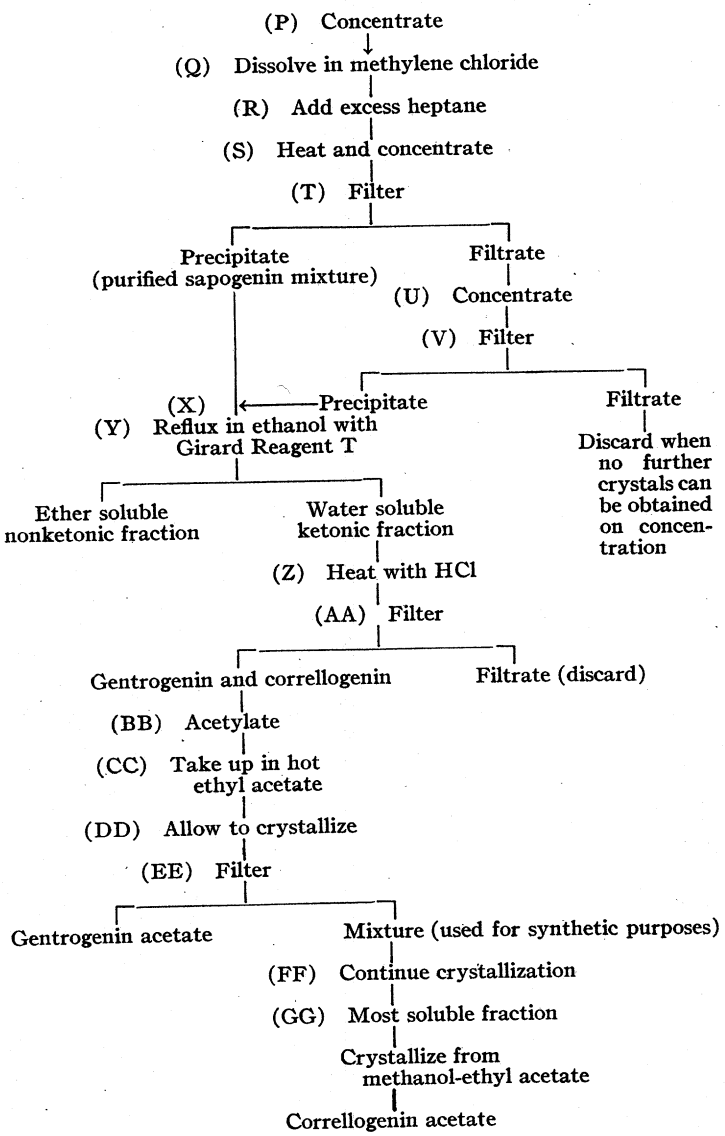


Fig. 1.—Flowsheet for isolation and purification of gentrogenin and correllogenin. Letters on flowsheet correspond to similar letters in text.

respectively (4). However, the properties of the new sapogenins differ greatly (2, 3) from those reported by Marker (4). It is the object of this paper to report additional information on the occurrence and isolation of gentrogenin and correllogenin.

OCCURRENCE

To date gentrogenin and correllogenin have been found only in the tubers of *Dioscorea spiculiflora*. This species has been found in Chiapas province of Mexico near the Guatemalan border. While it may occur in other regions of Mexico or Central America, results of extensive surveys in our laboratory indicate that it cannot be of common occurrence in other areas (5–8). Table I gives the data on 12 samples of *D. spiculiflora*.

The total sapogenin content varied from 2.0–5.5%, moisture free basis, the average being 3.5%. The ketonic fraction varied from 20–55% of the total sapogenin, the average being approximately 40%. The nonketonic fractions consisted of diosgenin and yamogenin, the 12 desoxyanalogs of gentrogenin and correllogenin, respectively. Gentrogenin and diosgenin were predominant in the ketonic and nonketonic fractions, respectively. Thus the sapogenins in *D. spiculiflora* consist invariably of a mixture of

12 desoxy- and 12 keto-sapogenins with the 20α, 25D side chain predominant and a minor proportion of the respective 20α, 25L isomers. A review of side chain stereochemistry and nomenclature can be found in a recent paper (9).

Of interest is the fact that configuration of the side chain in *Dioscorea* seems to be species specific. Thus diosgenin (25D) is the sole constituent of the commercially important *D. composita;* in the equally important *D. floribunda* a mixture of diosgenin and yamogenin (25L) is found; similar side chain mixtures occur in *D. spiculiflora;* and in *D. bartletti* yamogenin is sometimes the sole constituent.

TABLE I.—OCCURRENCE OF GENTROGENIN AND CORRELOGENIN IN DIOSCOREA SPICULIFLORA

EURB No.	Date	Location (Chiapas)	Total Sapogenin % MFB	Ketonic Fraction %
3134	10/52	Cerro Juarez	3.2	30
3140	10/52	Tuxtla Gutierrez	2.0	25
3513	6/53	Tuxtla Gutierrez	3.0	20
5317	3/55	Cristobal	3.6	35
5767	12/55	Chiapa	3.7	30
5768	"	Tuxtla Gutierrez	5.5	50
5769	"	Tuxtla Gutierrez	4.7	55
5770	"	Oaxaca	3.8	40
5771	"	Oaxaca	3.0	35
5772	"	Oaxaca	2.0	45
5773	"	Oaxaca	5.0	55
5792	"	Tuxtla Gutierrez	2.9	35

ISOLATION OF GENTROGENIN AND CORRELLOGENIN

Figure 1 gives an outline of the processes used.

Extraction of Saponins.—The following procedure was typical. *Dioscorea spiculiflora* tubers, 27.6 Kg., 68% moisture, were ground to pass a one-inch screen on a Ball and Jewell[1] cutter (A). The ground tubers were immediately extracted with 60 liters of 95% isopropyl alcohol in a 50-gallon steam-jacketed kettle equipped with agitator and reflux condenser. After refluxing one hour, the mixture was cooled and the alcohol run off. The extraction was repeated twice (B). The marc was discarded and the extracts combined and concentrated at atmospheric pressure to a volume of 18.2 liters (C).

Purification of Saponins.—The essentially aqueous suspension thus obtained was adjusted to pH 4.0 with concentrated hydrochloric acid and 1.0 Kg. of technical sodium chloride added. These additions repress the solubility of the saponin in the aqueous phase. The aqueous suspension was extracted seven times with *n*-butanol using 3 liters for each extraction. The aqueous fraction was discarded and the combined butanol extracts washed once with one-fourth volume of 5% sodium chloride solution. The sodium chloride solution was extracted with an equal volume of butanol and then discarded, the butanol being added to the original butanol solution. For large-scale extraction we used a glass-lined kettle provided with an agitator and a sight glass. Alternatively large separatory funnels can be used dividing the original aqueous extract in batches of appropriate volumes.

The combined butanol extracts (24.6 liters) containing the purified *Dioscorea* saponins were placed in a 10-gallon still and 13.8 liters of water added. The constant boiling water-butanol mixture was distilled until all the butanol had been removed, leaving 7.4 liters of an aqueous suspension of purified saponins (D).

Hydrolysis of Saponins.—To the above aqueous suspension was added 2.5 liters of ethanol and 2.5 liters of 9.9 *N* hydrochloric acid making the final acidity 2 *N*. The acidic solution was refluxed 4 hours (E), cooled, and filtered (F). The filtrate was discarded; the precipitate was washed well with water.

Alkaline Purification of Sapogenins.—The precipitate thus obtained, consisting of a mixture of sapogenins, resins, and tars, was refluxed 15 minutes in a mixture of 2 liters of methanol containing 400 Gm. of potassium hydroxide and 6 liters of benzene (G). After cooling the solution was filtered (H) and the precipitate saved. To the filtrate was added 2 liters of water (I) and the aqueous layer was withdrawn. Both the benzene and aqueous fractions were saved (J). The precipitate, described above, was extracted twice with 4 liters of benzene each time (K) and then discarded. The benzene was used to re-extract the above aqueous layer (L), the latter then being discarded. All the benzene extracts were then combined and the solvent distilled, leaving 541 Gm. of crude sapogenin mixture (M). The alkaline treatment removes much tar and resin of acid or phenolic nature.

Purification of Sapogenins.—The crude sapogenins were dissolved in 5 liters of technical chloroform (N) and passed through 1.0 Kg. of Florisil (0) in a 4-inch diameter column. The adsorbent was then eluted with 15 liters of chloroform and the combined eluates concentrated to dryness (P). The residue was dissolved in 2.5 liters of methylene chloride (Q). This solution was mixed with 7.5 liters of heptane (R) and heated. The solution was then concentrated to 6.0 liters and allowed to stand (S). The crystalline precipitate which formed was filtered and washed with cold heptane (T). The filtrate was concentrated to 3.0 liters (U), the precipitate again filtered (V), and this process was repeated until only a syrup could be obtained. The yield of crystallized sapogenin mixture was 321 Gm. or 3.7% of the tubers, moisture free basis (X). Infrared analysis showed that 30% of the sapogenin mixture was ketonic with gentrogenin predominant.

Separation of Ketonic Fraction.—The purified sapogenins were dissolved in 3 liters of hot, absolute ethanol containing 300 ml. of glacial acetic acid. In order to separate the ketonic and nonketonic fractions 180 Gm. of Girard Reagent T was added to this solution and the mixture refluxed one-half hour (Y). The solution was cooled and neutralized with 3 liters of ice cold saturated sodium carbonate solution containing 200 Gm. of sodium carbonate. This mixture was extracted six times with ether, using 2 liters each time. The combined ethereal fractions were washed six times using 2 liters of water

each time. In this manner the ether-soluble nonketonic fractions were separated from the water-soluble Girard T derivatives of the ketonic sapogenins. In order to hydrolyze the Girard T derivatives and thus obtain the original sapogenins all the aqueous fractions plus the sodium carbonate solution were combined and acidified to pH 1.0 with concentrated hydrochloric acid (Z). The mixture was heated on the steam bath one hour, cooled and filtered (AA). The precipitate was washed well with water and all the filtrates discarded. The precipitate was dried *in vacuo* at 80° for 10 hours, and weighed 92 Gm. Infrared analysis indicated that it was 100% ketonic sapogenin.

Separation of Gentrogenin and Correllogenin.—The above ketonic fraction was converted to the acetate by refluxing 2 hours with 450 ml. of acetic anhydride (BB). The acetic anhydride was removed by distillation *in vacuo* and the last traces removed by drying in a vacuum oven at 80°. The mixture was refluxed with 1.5 liters of ethyl acetate (CC) and allowed to stand at room temperature (DD). The long rectangular crystals which formed were filtered (EE). This product was pure gentrogenin acetate, yield 25.0 Gm. The mother liquors were concentrated, allowed to crystallize, and the process repeated until no more crystals were obtained (FF). Most of these fractions were mixtures of gentrogenin and correllogenin acetates. They are useful for synthetic purposes as they become identical on side chain degradation. The last and most soluble fractions were enriched in correllogenin acetate. Repeated crystallization from a 1-1 mixture of methanol-ethyl acetate gave 0.5 Gm. of pure correllogenin acetate (GG).

Physical Properties.—The physical properties of the new sapogenins are as follows: gentrogenin, m. p. 215–216°, $[\alpha]_D^{25}$ −57°; gentrogenin acetate, m. p. 227°, $[\alpha]_D^{25}$ −56°; correllogenin, m. p. 209–210°, $[\alpha]_D^{25}$ −69°; correllogenin acetate, m. p. 213–214°, $[\alpha]_D^{25}$ −60°.

Infrared Spectra.—One of the most useful properties for the detection of correllogenin and gentrogenin in mixtures is the infrared spectra of these compounds. Figure 2 gives the infrared spectra of gentrogenin acetate (top) and correllogenin acetate (bottom). The strong band at 1713 cm.$^{-1}$ slightly stronger than the 1737 cm.$^{-1}$ acetate band is characteristic of gentrogenin and correllogenin acetates and is assigned to 12 ketones in steroids (10). This band is of approximately equal intensity in both compounds and hence is particularly useful in calculating the percentage of ketonic sapogenins found in mixtures with nonketonic sapogenins. Several bands which are useful in determining the percentage of correllogenin or gentrogenin in mixtures of the two are found near 900 and 920 cm.$^{-1}$ In the case of correllogenin and similar 25L sapogenins the 920 band is strong and the 900 band weak. The reverse is true in the case of gentrogenin and other 25D sapogenins. It is best to make a series of known mixtures and then determine the absorptivities at these two regions (11). Then the percentages of correllogenin or gentrogenin may be approximated.

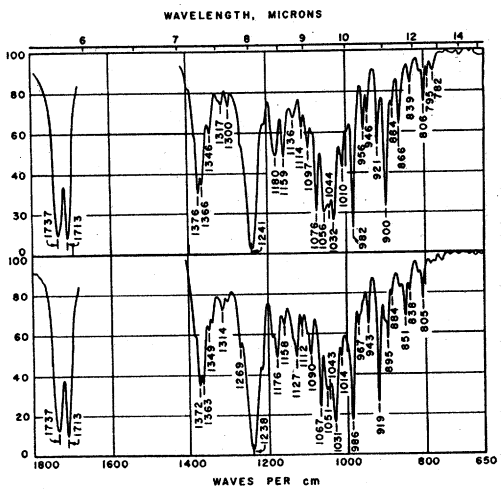


Figure 2.

SUMMARY

1. Gentrogenin and correllogenin, isomeric 12-ketonic sapogenins, have been isolated from tubers of *D. spiculiflora* with yields varying from 2.0 to 5.5 per cent total sapogenin and the ketonic fraction ranging from 20 to 55 per cent of the total sapogenin.

2. Gentrogenin and correllogenin were isolated by extraction and purification of their saponins followed by acid hydrolysis to the crude sapogenin. The sapogenins were purified by alkaline treatment followed by chromatography and crystallization. Gentrogenin and correllogenin were separated from nonketonic sapogenins (diosgenin and yamogenin) by treatment with Girard's reagent T and were then acetylated. Crystallization from ethyl acetate gave the insoluble gentrogenin acetate. Correllogenin in pure form was obtained from the soluble mother liquors. The more important physical constants including melting point, optical rotation, and infrared spectrum are presented.

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